Lithium and Transition Metal Ions Enable Low Energy Collision-Induced Dissociation of Polyglycols in Electrospray Ionization Mass Spectrometry

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Electrospray ionization tandem mass spectrometry has the potential to be widely used as a tool for polymer structural characterization. However, the backbones or molecular chains of many industrial polymers including functional polyglycols are often difficult to dissociate in tandem mass spectrometers using low energy collision-induced dissociation (CID). We present a method that uses Li^+ and transition metal ions such as Ag^+ as the cationization reagents for electrospray ionization in an ion trap mass spectrometer. It is shown that lithium and transition metal polyglycol adduct ions can be readily fragmented with low energy CID. Comparative results from different cationization reagents in their abilities of producing both MS spectra and CID spectra are shown. This method opens the possibility of using conventional and readily available low energy CID tandem MS to study polyglycol structures. (J Am Soc Mass Spectrom 2001, 12, 832–839) © 2001 American Society for Mass Spectrometry

Mass spectrometry (MS) has become an increasingly important tool for polymer analysis. In principle, structural information can be obtained by using tandem MS or MS/MS where the oligomer ion of interest is selected for dissociation via techniques such as collision-induced dissociation (CID) [1]. Tandem MS combined with chemical ionization, field desorption, secondary ion, or fast atom bombardment has been used to analyze short chain oligomers [2–17]. With the development of electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), high molecular weight polymers become readily amenable to MS analysis. While ESI and MALDI tandem MS have been widely used for biomolecule structural analysis, their use in polymer structural analysis is presently limited in scope [18–29]. One major reason is that many industrially important polymers do not readily fragment under the low energy CID condition [18–21, 28]. These polymers require the usage of high energy CID that can only be done in special instruments such as a sector/time-of-flight mass spectrometer [18–21, 28]. Unfortunately this type of instrument is not accessible to most researchers for routine analyses. MALDI in-source or post-source decay can generate fragment ions for certain polymers [18, 30–33]; but the general applicability of the technique and quality of the fragment ion spectra for structural analysis remain to be determined. On the other hand, there are a host of ESI mass spectrometers with different instrumental configurations that can provide low energy CID capability. It is clear that a robust and readily adaptable method for generating low energy CID spectra of polymeric materials would open the door for many polymer researchers to characterize their polymers by MS/MS.

One class of polymers that attract much attention in the polymer industry are functional polyglycols, including poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG) and their copolymers [34, 35]. Structural characterization of functional polyglycols is important because polyglycols are widely used in industry with their properties depending on structures and compositions, and polyglycols are being increasingly used for biotechnical and biomedical applications such as the development of slow releasing drugs [34, 35]. High energy CID of polyglycols in combination with secondary ion or fast atom bombardment (FAB) [17] and MALDI [18] has been investigated. Of interest, it has been shown [17] that all alkali adduct ions of PEG can fragment by high energy CID; but the Li^+ adduct ion gives somewhat enhanced fragment ion signals, compared to Na^+ or K^+ adducts. Under the low energy CID condition, the Na^+ or K^+ PEG adduct ions do not fragment in conventional tandem mass spectrometers [17, 21]. There is one report showing the fragmentation of $[PEG+Na]^+$ in MALDI FTMS (Fourier transform mass spectrometry) using sustained off-resonance irradiation CID [22]. Lattimer

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has shown that lithiated polyglycol ions generated by FAB can undergo efficient low energy CID to produce information rich MS/MS spectra for low mass oligomers ($m/z < 500$) [14].

There are a number of reports of ESI MS studies of polyglycols [36–54]. In general, sodiated or potassiated PEG adducts formed by ESI do not dissociate in the interface region [36–54]. In contrast, protonated PEG fragments so easily that in-source fragmentation cannot be avoided [21]. Moreover, protonation is generally not very efficient compared to alkali adduct ion formation. Thus, protonated PEG signals are either absent or weak in the mass spectrum of PEG, even with strong acidification of sample solutions. This is particularly true for many real-world samples where the concentration of alkali ions is high. Even with extensive sample cleanup, the residual alkali ions can still suppress the protonation of PEG. In short, functional polyglycols are currently viewed as a class of polymer that is not readily amendable to ESI low energy CID for structural characterization.

In this work, we report a robust and routine method of generating low energy CID mass spectra of polyglycols in an ESI ion trap mass spectrometer. Based on the work of Lattimer on FAB CID MS studies of polyglycols [14], we demonstrate herein that lithiated polyglycols formed by ESI can be readily fragmented with low energy CID and, with ESI, we demonstrate the possibility of generating fragment ions for oligomers with masses around 3100. In addition, we find that polyglycol adduct ions with transition metal ions can also be readily fragmented in a manner from which rich structural information can be obtained.

Experimental

Materials and Reagents

In our experiment, poly(ethylene glycol) (hydroxyl terminated), poly(ethylene glycol) methyl ether, poly(ethylene glycol) butyl ether, poly(propylene oxide) (hydroxyl terminated), and poly(propylene oxide) butyl ether, with average molecular weights ranging from 300 to 4000 Da, were studied. Unless indicated otherwise, all samples were purchased from Aldrich Chemical Co. (Milwaukee, WI) and analyzed without purification. Representative results from PEG methyl ether (average M_n ca. 550), PEG (average M_n ca. 3350) and PPG (average M_n ca. 2000) are presented in this paper. Phosphoric acid stock solution, ammonium acetate, sodium chloride, lithium chloride, silver nitrate, cobalt chloride, nickel nitrate and zinc chloride were obtained from various commercial sources and used as received. High performance liquid chromatography (HPLC) grade methanol was obtained from Sigma (Milwaukee, WI). Distilled water was from a Milli-Q UV plus ultra-pure system (Millipore, Mississauga, ON).

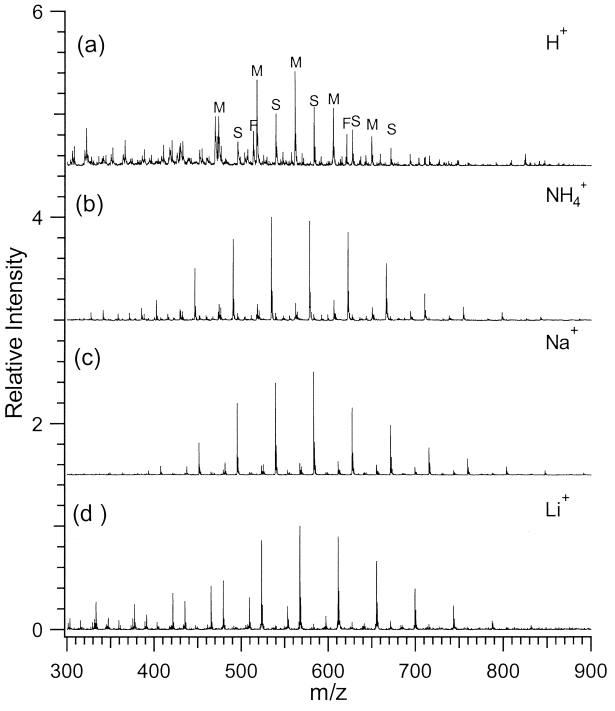


Figure 1. ESI mass spectra of PEG methyl ether obtained using different cations for ionization: (**a**) H^+, (**b**) NH_4^+, (**c**) Na^+, and (**d**) Li^+.

ESI MS and MS/MS

Phosphoric acid solution was diluted and all salts were dissolved in water at a concentration of 0.1 M. The analyte solution was prepared by mixing PEG or PPG stock solution, 10% (v) cationization solution and appropriate amount of water/methanol mixture (volume ratio 1:1 for PEG and 1:3 for PPG) to make the PEG or PPG final concentration of about 100 μM.

All MS experiments were carried out in a Bruker/Agilent Esquire-LC Ion Trap LC/MS^n system. Sample solution was infused into the electrospray interface by a syringe pump (Cole-Parmer Instrument Co., IL) at a flow rate of 10 μl/min. The potentials between needle and capillary, capillary and skimmer 1, were 3.5 KV and 50 V, respectively. The capillary temperature was kept at 300 °C. Mass spectra were acquired over the range of m/z 15–2200. All data were reprocessed using the Igor Pro Software package (WaveMetrics, Lake Oswego, OR) without background subtraction.

Results and Discussion

Figure 1 shows the ESI spectra of PEG methyl ether (average M_n *ca.* 550 Da) obtained using different cationization reagents. Despite great effort to minimize in-source fragmentation by lowering the voltage differences between source and skimmer, and improving signal-to-noise (S/N) ratio by adjusting solvent conditions, protonation of PEG generates the most complex and noisy spectrum (Figure 1a). Protonated species (M

as labeled in Figure 1a), sodiated species (S), and some in-source fragments (F) are identified. In the NH_4^+ case, ammoniated, protonated, sodiated species, and fragment ions are observed (Figure 1b); but the in-source fragmentation is less severe compared to the protonation experiment. The ESI mass spectra of Na^+, K^+, and Cs^+ adduct ions are almost the same and Figure 1c displays the spectrum obtained from the sodium cationization of PEG. For all other polyglycols studied by ESI in this work, alkali ions provide efficient cationization with no in-source fragmentation.

Figure 1d shows the ESI mass spectrum obtained using lithium ions as the cationization reagent. It is interesting to note that the intensities of the minor components in the low mass region (<500 Da) are somewhat enhanced in the Li^+ case. This finding is similar to what was observed in MALDI analysis of low mass polyglycols using Li^+ as the cationization reagent [55]. Cationization of PEG and other polyglycols by alkali metal ions including Li^+, Na^+, K^+, and Cs^+ has been extensively studied using MALDI to produce the adduct ions [55–57]. The observation of more intense low mass oligomers generated using Li^+ compared to Na^+ in MALDI was attributed to the fact that the lowest energy conformer of $[PEG+Li]^+$ requires less oxygen atoms than for $[PEG+Na]^+$ [57]. The same explanation may be applied to this ESI work. The implication of this observation for polymer molecular weight analysis is quite obvious, but is not the focus of this paper.

In practice, Na^+ is the most common contaminant in PEG analysis. Na^+ can be brought into a sample from polymer initiators, solvents, and/or containers used for sample preparation. It is not surprising that most of the PEG spectra obtained by ESI and MALDI are sodiated peaks, even if no cationization reagent is purposely added to the sample. For molecular weight analysis where ion fragmentation must be avoided, Na^+ serves the purpose of generating reproducible mass spectra of PEGs. Unfortunately, these easily formed $[PEG+Na]^+$ ions fail to produce fragment ions under low energy CID in the ion trap mass spectrometer. The K^+ and Cs^+ adduct ions do not fragment either.

As shown in Figure 1d, Li^+ can provide efficient ionization of polyglycols to produce intact oligomer ions. Moreover, ESI generated Li^+ adduct ions can be readily fragmented using low energy CID, as illustrated in Figure 2c. This observation is entirely consistent with what Lattimer has reported in the FAB low energy CID experiment [14]. Compared to other alkali metal ions, Li^+ was found to enhance the fragmentation of polyglycols [14] as well as a variety of other compounds [58–60]. While the ESI-generated ions generally possess far less internal energy (i.e., cooler ions) than those produced by FAB or MALDI [61], our work suggests that the energy gained through CID is sufficient to fragment the lithiated ESI ions.

Figure 2 shows the ESI MS/MS spectra of the PEG 11-mer produced using three different cationization reagents. While protonation is not an effective way of

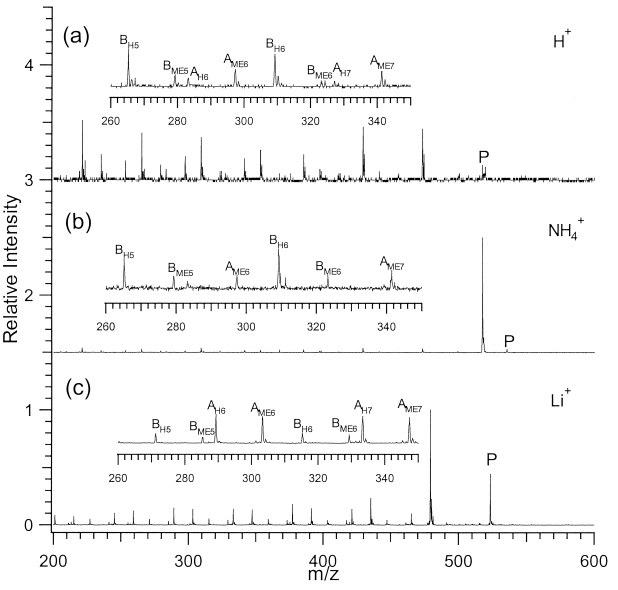
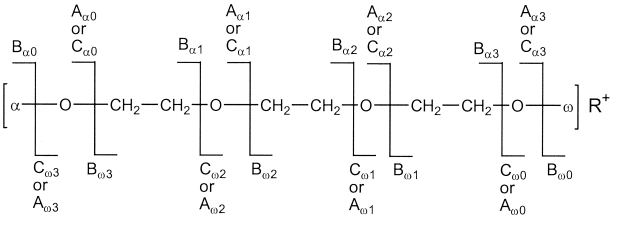


Figure 2. ESI MS/MS spectra of PEG methyl ether 11-mer with different cations: (**a**) H^+, (**b**) NH_4^+, and (**c**) Li^+.

generating MS spectra, $[PEG+H]^+$ ions, if present, can be fragmented as shown in Figure 2a. This is not surprising in light of the fact that in-source fragmentation can be readily observed in protonated PEG species. Since the signal intensity in the MS spectrum of $[PEG+H]^+$ is low, the MS/MS spectrum of $[11\text{-mer}+H]^+$ shown in Figure 2a also displays a low S/N ratio.

In the case of NH_4^+ cationization (Figure 2b), the most dominant peak in the MS/MS spectrum is the protonated 11-mer as a result of the loss of NH_3 (-17 Da) from the molecular ion peak. Some weak fragment peaks from subsequent fragmentation of $[11\text{-mer}+H]^+$ are also observed (see the inset of Figure 2b). Compared to panels a and b in Figure 2, the CID spectrum of $[11\text{-mer}+Li]^+$ (Figure 2c) shows a higher S/N ratio. This is also true for other polyglycols studied in this work. The fragment ions observed correspond to backbone chain cleavage and can be readily assigned to the polyglycol structures. Scheme **1** shows the fragmentation pattern of a singly charged oligomer (trimer) ionized by a cationization reagent R. The nomenclature for the fragment ions is consistent with that reported [14, 17]. To differentiate fragment ions containing different end groups, α and ω are used to represent ions contain-



Scheme 1

ing α and ω end group, respectively. The number in the label denotes the nth repeat unit from the end group α or ω. The A series ions are likely generated by the charge-induced fragmentation mechanism, where the B and C series ions are most likely formed via charge-remote fragmentations [14, 17]. For PEG methyl ether, α is hydrogen (H) and ω is the methyl group (ME). Some of the fragment ions shown in Figure 2 are labeled according to Scheme **1**.

As Figure 2 shows, the fragment ions from protonated or lithiated precursor ions are distributed throughout the entire mass range. This observation is somewhat different from what was found in FAB MS/MS. In FAB, the MS/MS spectrum of the lithiated PEG decamer ion showed intense fragment ions throughout the entire mass range, whereas the fragment ions from the protonated decamer ion showed only low mass fragment ions [14]. The difference observed in ESI MS/MS and FAB MS/MS is likely due to the difference in energetics for the precursor ions generated by the two modes of ionization. FAB generates ions with higher internal energy, compared to ESI [61]. Protonated species are more labile than the lithiated ions and the additional energy gained via CID in FAB can cause a greater extent of fragmentation for the protonated ions, resulting in predominately low mass fragment ions in FAB MS/MS of $[\text{decamer}+\text{H}]^+$. Figure 2a or b also shows that the B_H series ions are relatively more intense in the MS/MS spectra of protonated 11-mer, compared to the B_{ME} and A series ions. This is likely due to the readiness of protonation of the hydroxyl end group. As a consequence, charge-remote fragmentation from the hydroxyl end is favored. For the fragmentation of lithiated 11-mer ions (Figure 2c), the A_H and A_{ME} series ions show similar peak intensity and both are stronger than the B series ions. Charge-induced fragmentation is clearly favored and peak intensities of both A and B series ions are not affected by the end group structure. This observation is not surprising considering that, unlike protonation, the lithium ion forms a complex with multiple oxygens in the ethylene oxide chains. Note that FAB MS/MS of lithiated species also showed more intense A series ions generated by the facile process of charge-induced fragmentation [14].

The ability of lithiated polyglycol ions to dissociate under low energy CID is related to the binding strength between the metal ion and polyglycol chain [14, 59]. The binding between Li^+ and the polymer chain is stronger than that between the other alkali metal ions and the polymer chain [14]. Thus the internal energy gained via CID, in the case of lithiated oligomer ions, is channeled to the polymer chain, causing polymer dissociation. In contrast, for the other alkali metal ions, dissociation of the interactions between the metal ion and polymer chain is the energy release channel. It is clear that any metal ions bound strongly to the polymer chain would potentially cause fragmentation of the polymer chain, provided that a sufficient amount of energy is given to the adduct ion. This is indeed the case for the polyglycol

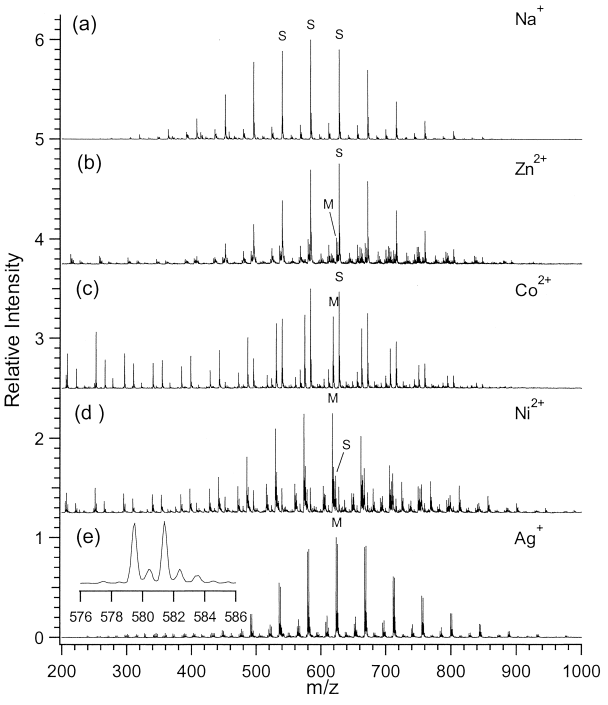


Figure 3. ESI mass spectra of PEG methyl ether obtained using sodium ion and different transition metal ions for ionization: (**a**) Na^+, (**b**) Zn^{2+}, (**c**) Co^{2+}, (**d**) Ni^{2+}, and (**e**) Ag^+.

complex formed by using divalent transition metal ions as the cationization reagents in ESI.

Figure 3 shows the ESI mass spectra of PEG methyl ether obtained using transition metal ions for ionization. Panels b, c, and d in Figure 3 show the spectra obtained with Zn^{2+}, Co^{2+}, and Ni^{2+}, respectively. The oligomer peaks labeled as M are from $[\text{oligomer}+\text{metal}-\text{H}]^+$. Apparently, in ESI, the divalent metal ion reacts with the oligomer to form the singly charged species with the loss of a proton. The sodiated ESI spectrum is shown in Figure 3a for comparison. Despite the use of a large amount of salts (~100 mM), the ESI spectra from Zn^{2+}, Co^{2+}, and Ni^{2+} still display peaks from sodiated species with varying relative intensity (labeled as S in Figure 3). This observation suggests that these metal ions do not ionize the polyglycol as efficiently as the alkali metal ions. However, their adduct ions can be readily fragmented under low energy CID, as shown in panels a, b, and c in Figure 4. By replacing a proton, the divalent metal ion forms a strong bond with the polymer chain and the energy gained by CID causes chain fragmentation.

The insets in Figure 4 show the expanded spectra with peaks labeled according to Scheme **1**. The A series ions containing the methyl end group are likely formed via charge-induced dissociation. This is illustrated in Scheme **2** using the ionization reagent Co^{2+} as an example. The metal ion is proposed to form a complex with the internal chains of ethylene oxide, which induces the loss of ethylene oxide group(s). The A and B series ions containing the hydrogen end group are

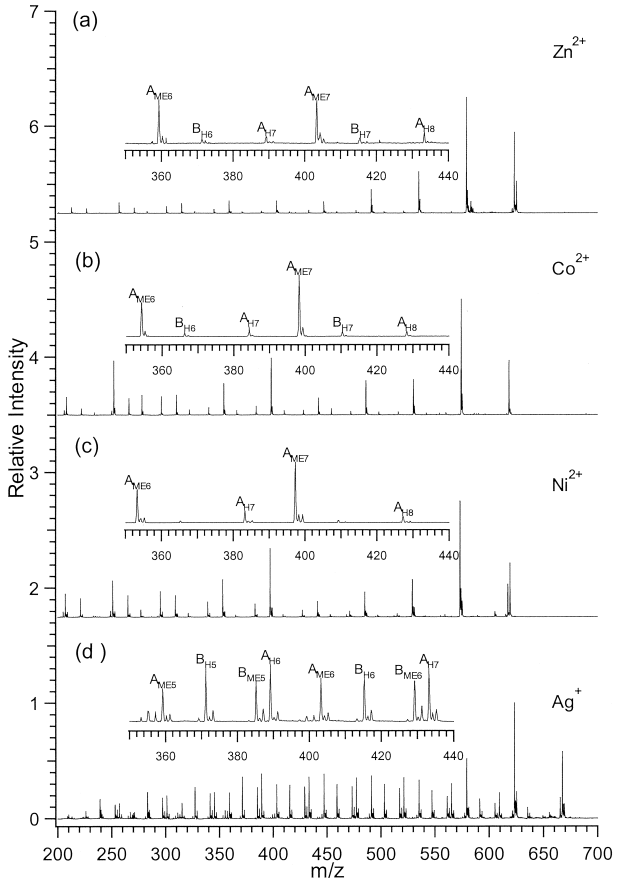
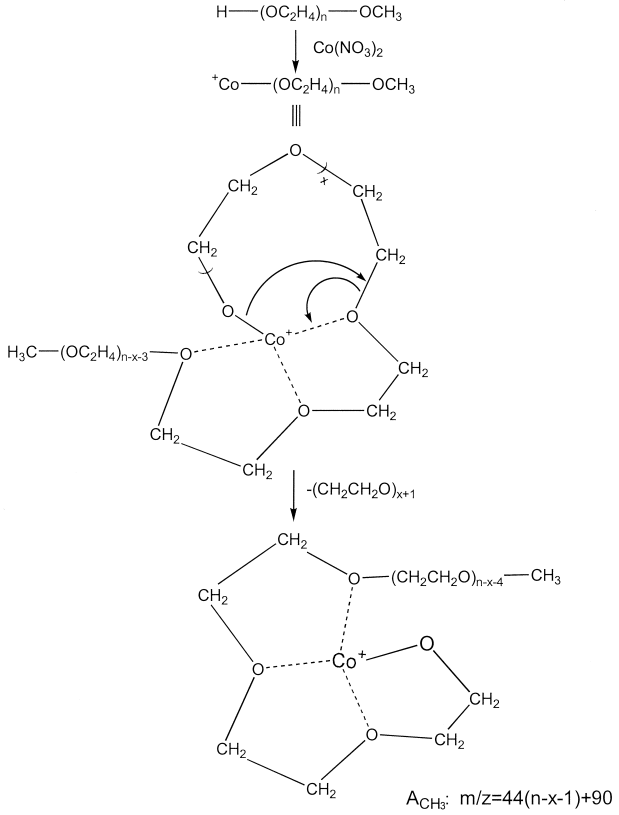


Figure 4. ESI MS/MS spectra of PEG methyl ether 11-mer with different transition metal ions: (**a**) Zn^{2+}, (**b**) Co^{2+}, (**c**) Ni^{2+}, and (**d**) Ag^{+}.

likely formed via charge-remote dissociation, as shown in Schemes **3** and **4**. The B series ions containing the methyl end group are not observed. This finding supports the argument of charge-remote dissociation responsible for generating the B series ions. The divalent metal ion is covalently bound to the hydroxyl end and thus charge-remote dissociation can only initiate from this site.

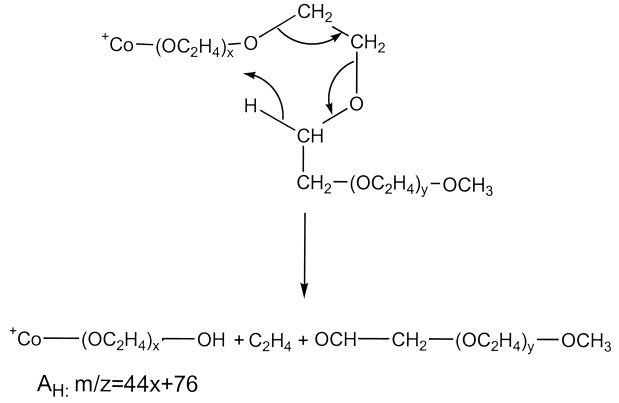
Another transition metal ion that we have investigated for ESI of polyglycols is the silver ion. Ag^{+} is known to be capable of ionizing non-polar polymers such as polystyrene in both ESI [62, 63] and MALDI [63] due to possible binding between Ag^{+} and phenyl rings. To our knowledge, there is no report on the use of Ag^{+} for ionization of polyglycols. To gauge the efficiency of silver cationization for PEG in ESI, a control experiment was conducted in which the same amounts of Na^{+}, Ag^{+}, and H^{+} were added to the PEG methyl ether sample. Sodiated and silver-ion attached peaks were detected while protonated species were completely suppressed. The intensity ratios between the Ag^{+}- and Na^{+}-adduct peaks vary from 1–3%. This result suggests that it should be possible to produce ESI spectra composed of predominately Ag^{+}-PEG adduct ions by adjusting the silver ion concentration. For all polyglycol



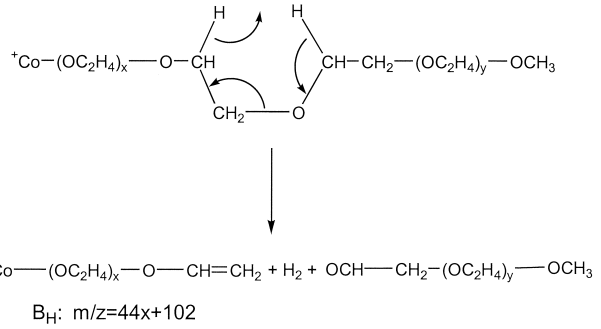
Scheme 2

samples listed in the Experimental and several real world samples [64], we can consistently obtain oligomer distributions with silver cationization. In contrast, most of these samples produce few or no protonated species, even in very acidic solvents for ESI. These results demonstrate that, besides alkali ions, silver cationization is also very effective for generating ESI spectra of polyglycols.

One unique feature of using Ag^{+} cationization for generating ESI mass spectra is the distinct isotope pattern associated with the silver adduct ion. Any singly-charged silver-attached oligomer ion displays two peaks, two mass units apart, with similar intensi-



Scheme 3



Scheme 4

ties. This is shown in the inset of Figure 3e. The isotope pattern greatly facilitates the peak assignment in the ESI mass spectrum. With alkali metal ions, we often need to use different metal ions for ionization and observe the mass shifts to confirm the peak assignment. This is particularly true for distinguishing peaks of the actual minor components in a sample from those resulting from different metal ions or proton attached to the principal polymer distribution. In the PEG methyl ether example, by examining the spectrum shown in Figure 3e, the minor peaks displaying the characteristic isotope peaks must be from impurities present in the sample.

The silver-attached adduct ions can be dissociated very efficiently under the low energy CID condition; yet they are sufficiently stable to survive the interface region. Figure 4d shows the fragment ion spectrum of the argentinated 11-mer. Compared to the MS/MS spectrum from the lithiated adduct ion (Figure 2c), a larger number of fragment ions with greater intensities are observed in Figure 4d. In addition, an MS/MS experiment can be readily conducted in a manner where the mass window of the parent ion is varied to include Ag^{107} isotope only, Ag^{109} isotope only, or both of them, respectively (data not shown). Any silver ion attached fragments display the isotope patterns, thereby facilitating the interpretation of fragment ions (e.g., any internal fragment ions with no silver ion attached will not show the isotope pattern). The fragment ions observed in Figure 4d are from the cleavage of polyglycol chains with two different end groups (−OH and −OMe). Peak assignment is shown in the inset of Figure 4d according to Scheme **1**. Compared to the fragmentation of lithiated adduct ions (Figure 2c), argentinated 11-mer gives much more intense B series ions. This is likely due to the formation of more stable B ions by silver attachment. For the B ion with a structure of $Ag[CH_2{=}CH-(OCH_2CH_2)_y-OH \text{ or } CH_3]^+$, the silver ion can interact with the double bond via d-π interaction to form a strong binding. In the case of lithium, only electrostatic interactions between the metal ion and the oxygens occur.

Poly(ethylene glycol) butyl ether (M_n ~1000) and poly(propylene glycol) butyl ether (M_n ~1100) were also examined by using silver ion as the cationization reagent. The ionization and fragmentation behavior of these two samples is similar to that of PEG methyl ether. In the MS/MS spectra (data not shown), the dominant fragment peaks are from the A and B series ions, as in the case of PEG methyl ether. No obvious steric hindrance effect on fragmentation from the butyl group is observed.

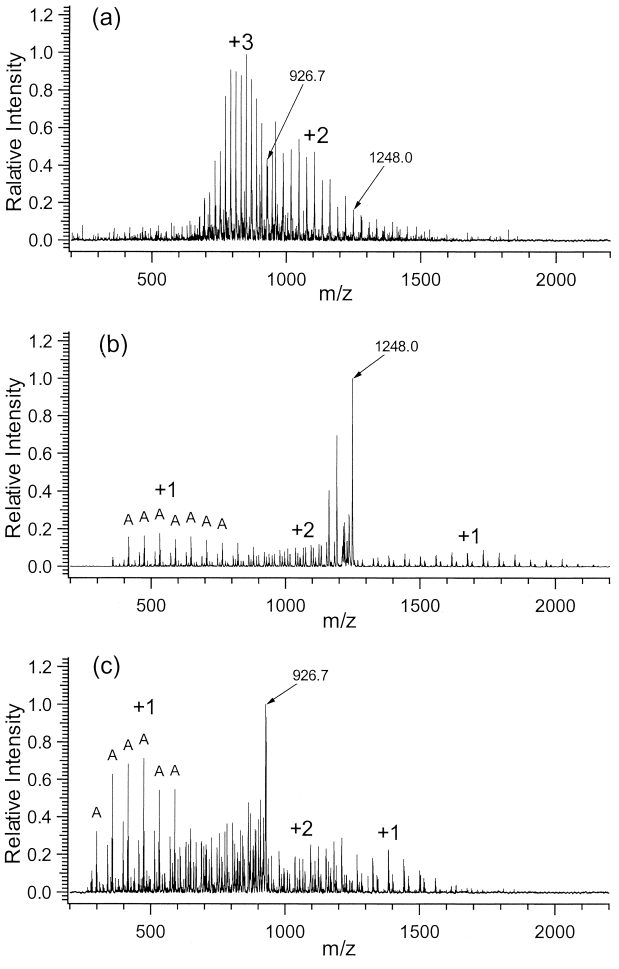


Figure 5. (**a**) ESI mass spectrum of PPG 2000 obtained using Ag^+ as the cationization reagent. (**b**) ESI MS/MS spectrum of the doubly charged ion at m/z = 1248.0. (**c**) ESI MS/MS spectrum of the triply charged ion at m/z = 926.7.

One important attribute of ESI is the ability to generate multiply charged ions, which are found to be more readily dissociated compared to the singly charged ions [37]. Thus ESI tandem MS can potentially be very useful for determining structures of higher mass polymers that are not amendable to FAB or even MALDI MS/MS. Figure 5 illustrates the ESI mass spectrum of PPG using Ag^+ as the cationization reagent. There are two distributions. Judged by mass spacing, the lower m/z range (600–1000) is composed of mainly triply charged species while the higher m/z range (1000–1500) consists of mainly doubly charged species. No singly charged species were detected. The relative intensities between two charge states could be greatly affected by varying the instrumental parameters, such as cone voltage and trap drive in the ion trap

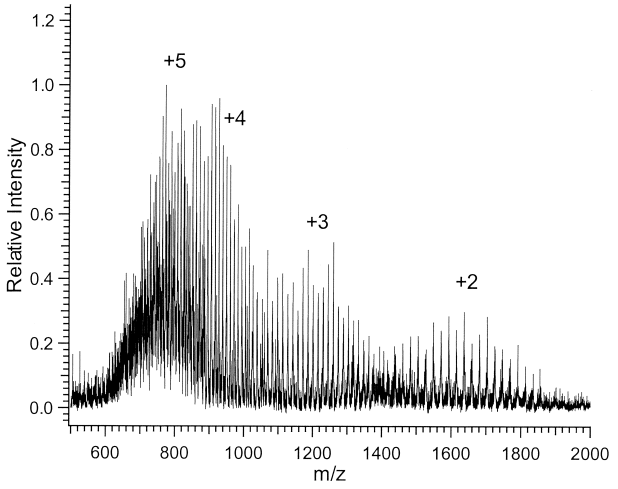


Figure 6. ESI mass spectrum of PEG 3350 obtained using Ag^+ as the cationization reagent.

mass spectrometer. Figure 5b shows the MS/MS spectrum of the doubly charged ions with *m/z* 1248.0. Two types of product ions can be identified: *m/z* lower than the parent ion and all the way to *m/z* ~700, there is a series of doubly charged product ions, and the peaks outside the *m/z* region 700–1200 are clearly from singly charged product ions separated by 58 units. Structure information can be obtained by analyzing the low mass, singly charged products. Some of the peaks corresponding to the A series ions of PPG are labeled in Figure 5b. Figure 5c is the MS/MS spectrum of a triply charged ion at *m/z* = 926.7. It bears a similar pattern to that of the doubly charged species, except that triply charged products are also found mixed with the doubly charged fragment ion products.

Figures 6 and 7 show examples of using silver ion as cationization reagent for generating MS and MS/MS spectra of polyglycols with masses around 3000. Figure 6 is the MS spectrum of PEG 3350 displaying oligomer peaks with multiple charges from +2 to +5. Figure 7a shows the fragment spectrum of the triply charged ion at *m/z* ~1171. Many charge stripping products and multiply charged fragment ion products are observed. The peak at *m/z* 1746 is likely due to an artifact associated with the ion trap instrument (i.e., leakage of ions during isolation and dissociation). Figure 7b shows the MS/MS spectrum from a +4 ion at *m/z* 927, displaying many fragment ions. Some of the peaks from the A and B series ions are labeled. Figure 7c shows the fragment ion spectrum of the +5 ion at *m/z* 727. Extensive fragment ions resulting from the polymer chain dissociation are observed. Again, the peak at *m/z* 1230 in Figure 7b, and the peak at *m/z* 905 in Figure 7c are likely the leaked ions from the intact oligomer ions. For the +5 ion at *m/z* 727, there are five silver ions attached to the oligomer. Thus the oligomer mass is about 3100.

Attempts to produce fragment ion spectra of polyglycols with molecular weights above 4000 so far have

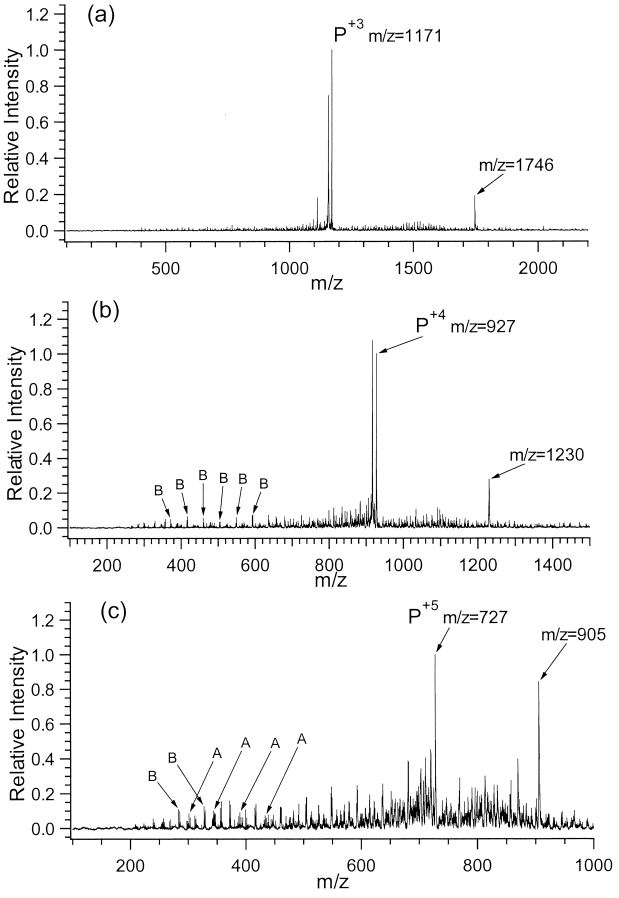


Figure 7. ESI MS/MS spectra of PEG 3350 with Ag^+ as the cationization reagent: (**a**) from +3 ion at *m/z* = 1746, (**b**) from +4 ion at *m/z* = 927, and (**c**) from +5 ion at *m/z* = 727.

failed to give any meaningful results. We found that the ESI spectra for the higher mass polyglycols are very complex. Isolation of a single oligomer ion is difficult with the current ion trap instrument. A systematic investigation of how experimental parameters affect the mass range assessable for MS/MS is currently underway.

In conclusion, we have shown that silver or lithium cationization is a facile method for generating low energy CID spectra of polyglycols. This method opens the possibility of studying polymer structures with conventional tandem mass spectrometers. We are currently expanding this work to PEG/PPG copolymers and other types of polymeric systems.

Acknowledgments

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